

Supplementary data

Terminalin from African Mango (*Irvingia gabonensis*) Stimulates Glucose Uptake through Inhibition of Protein Tyrosine Phosphatases

Sun-Young Yoon ^{1,†}, Jinsoo Kim ^{2,3,†}, Bum Soo Lee ^{2,†}, Su Cheol Baek ², Sang J. Chung ^{2,3,*} and Ki Hyun Kim ^{2,*}

¹ Department of Cosmetic Science, Kwangju Women's University, Gwangju 62396, Korea

² School of Pharmacy, Sungkyunkwan University, Suwon 16419, Republic of Korea

³ Department of Biopharmaceutical Convergence, Sungkyunkwan University, Suwon 16419, Republic of Korea

[†] These authors contributed equally to this work.

*Corresponding author. Phone: +82-31-290-7703 (S.J.C). E-mail address: sjchung@skku.edu (S.J.C)

*Corresponding author. khkim83@skku.edu (K.H.K.); Tel.: +82-31-290-7341 (C.P.); +82-31-290-7700 (K.H.K.)

Supporting Information Contents:

Materials and Methods: Western blotting	S3
Figure S1. HR-ESIMS data of terminalin	S4
Figure S2. ^1H NMR spectrum of terminalin ($\text{DMSO-}d_6$, 800 MHz).....	S5
Figure S3. ^{13}C NMR spectrum of terminalin ($\text{DMSO-}d_6$, 200 MHz).....	S6
Figure S4. HMBC spectrum of terminalin ($\text{DMSO-}d_6$).....	S7
Figure S5. D-HMBC spectrum of terminalin ($\text{DMSO-}d_6$).....	S8
Figure S6. UV spectrum of terminalin	S9
Figure S7. LC/MS chromatogram of the crude extract (detected at 230 nm).....	S10
Figure S8. Effects of terminalin on C2C12 muscle cells.....	S11
Table S1. LC-MS conditions for the crude extract.....	S12

Materials and Methods

Western blotting

Proteins were extracted using RIPA buffer (Sigma-Aldrich, Saint Louis, Missouri, USA) containing protease inhibitor cocktail and phosphatase inhibitor cocktail (Sigma-Aldrich). Next, the proteins were separated by electrophoresis on a 10% sodium dodecyl sulfate-polyacrylamide gel and transferred to a polyvinylidene fluoride (PVDF) membrane (Merck KGaA, Darmstadt, Germany) using a wet transfer system. The membranes were incubated overnight at 4°C with primary antibodies. The primary antibodies used were as follows: anti-total AMPK, anti-phosphorylated AMPK, anti-total Akt, anti-phosphorylated Akt (Cell Signaling Technology, Beverly, MA, USA), and anti- β -actin (AbFrontier, Seoul, South Korea). Next, the membranes were incubated with an anti-rabbit-IgG-horseradish peroxidase-conjugated secondary antibody (Santa Cruz Biotechnology). Antibody-antigen complexes were detected using enhanced chemiluminescence reagents (GE Healthcare Korea, Incheon, South Korea). Chemiluminescent images were acquired using a LuminoGraph II Imaging System (ATTO Corporation, Tokyo, Japan).

Figure S1. HR-ESIMS data of terminalin

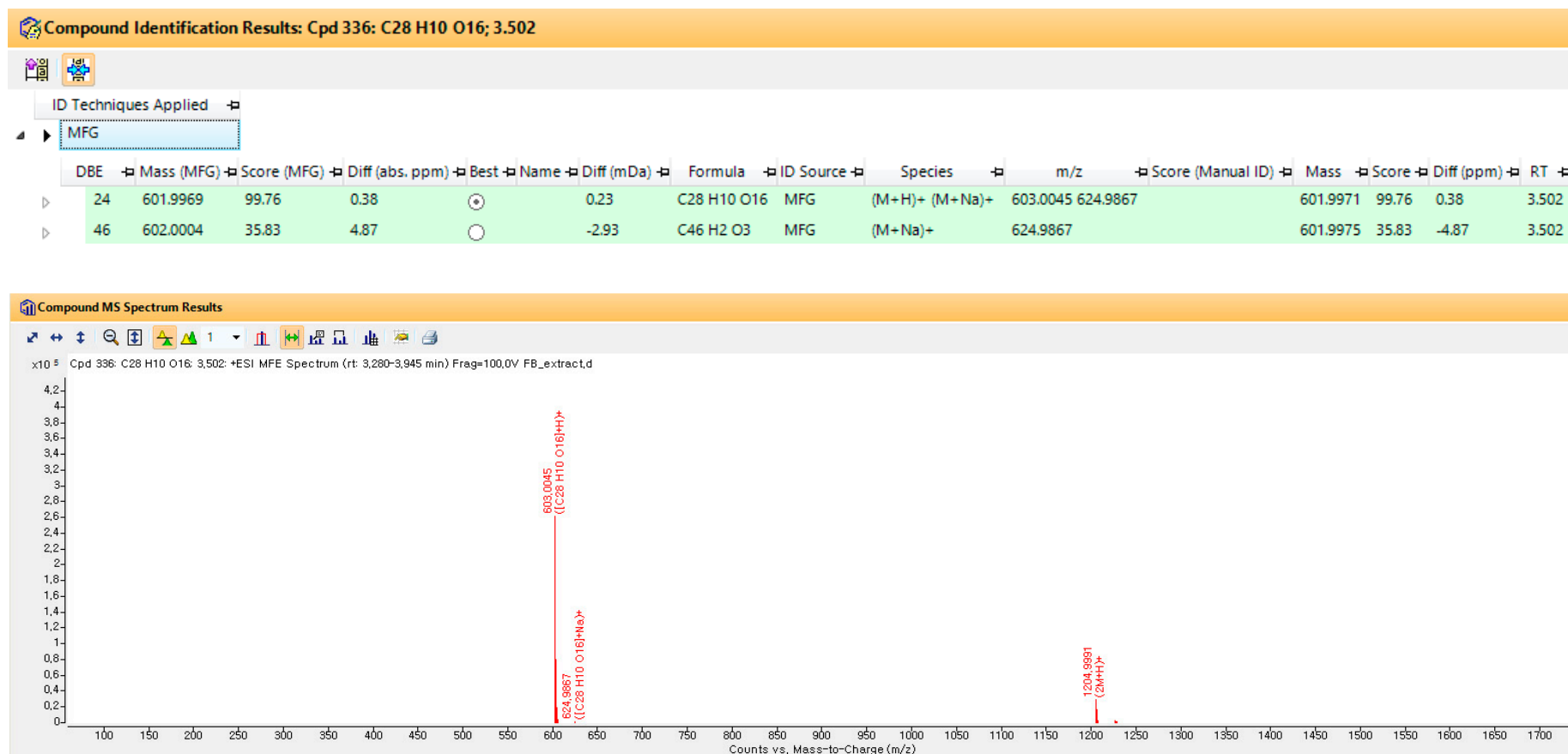


Figure S2. ^1H NMR spectrum of terminalin (DMSO- d_6 , 800 MHz)

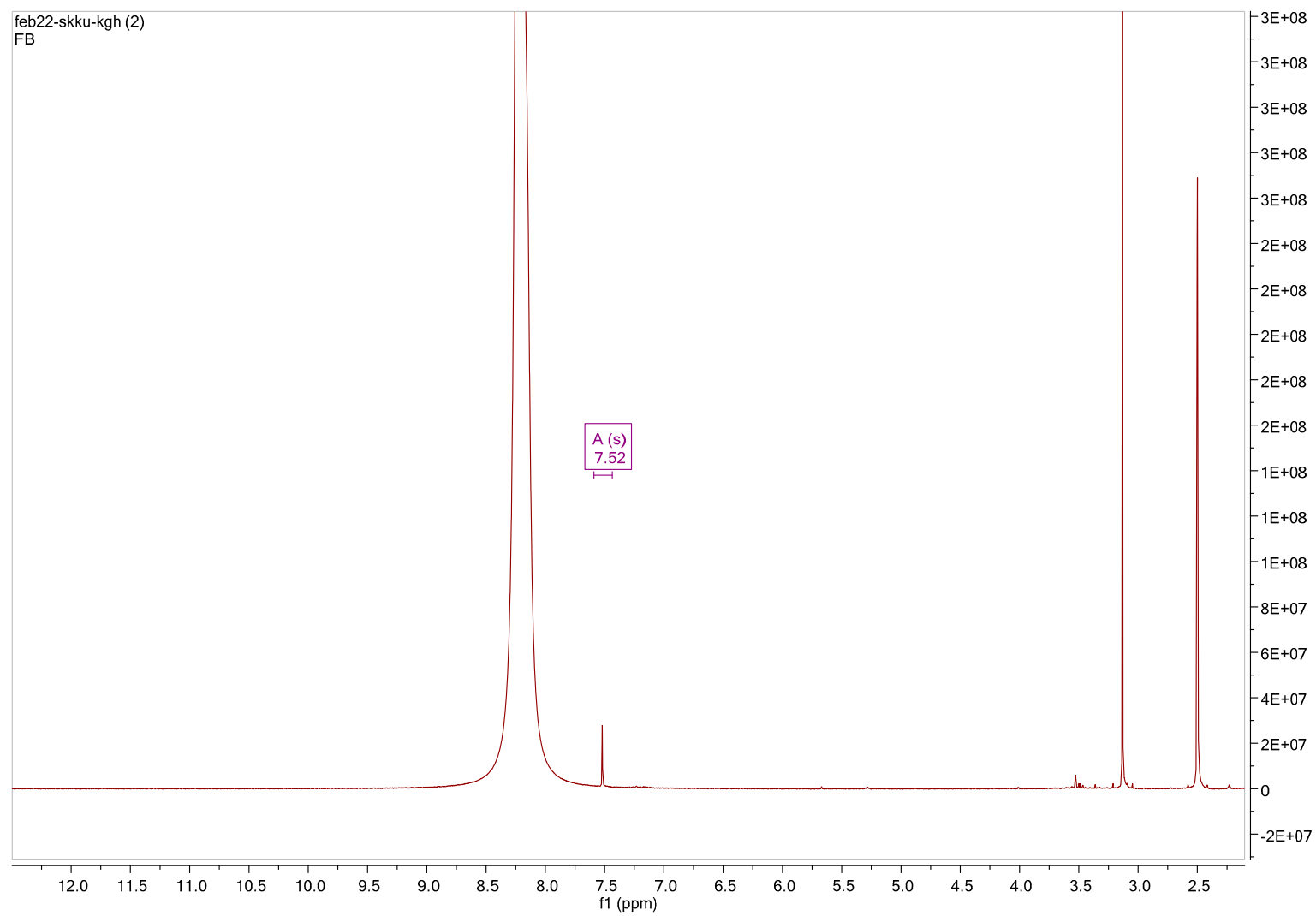


Figure S3. ^{13}C NMR spectrum of terminalin (DMSO- d_6 , 200 MHz)

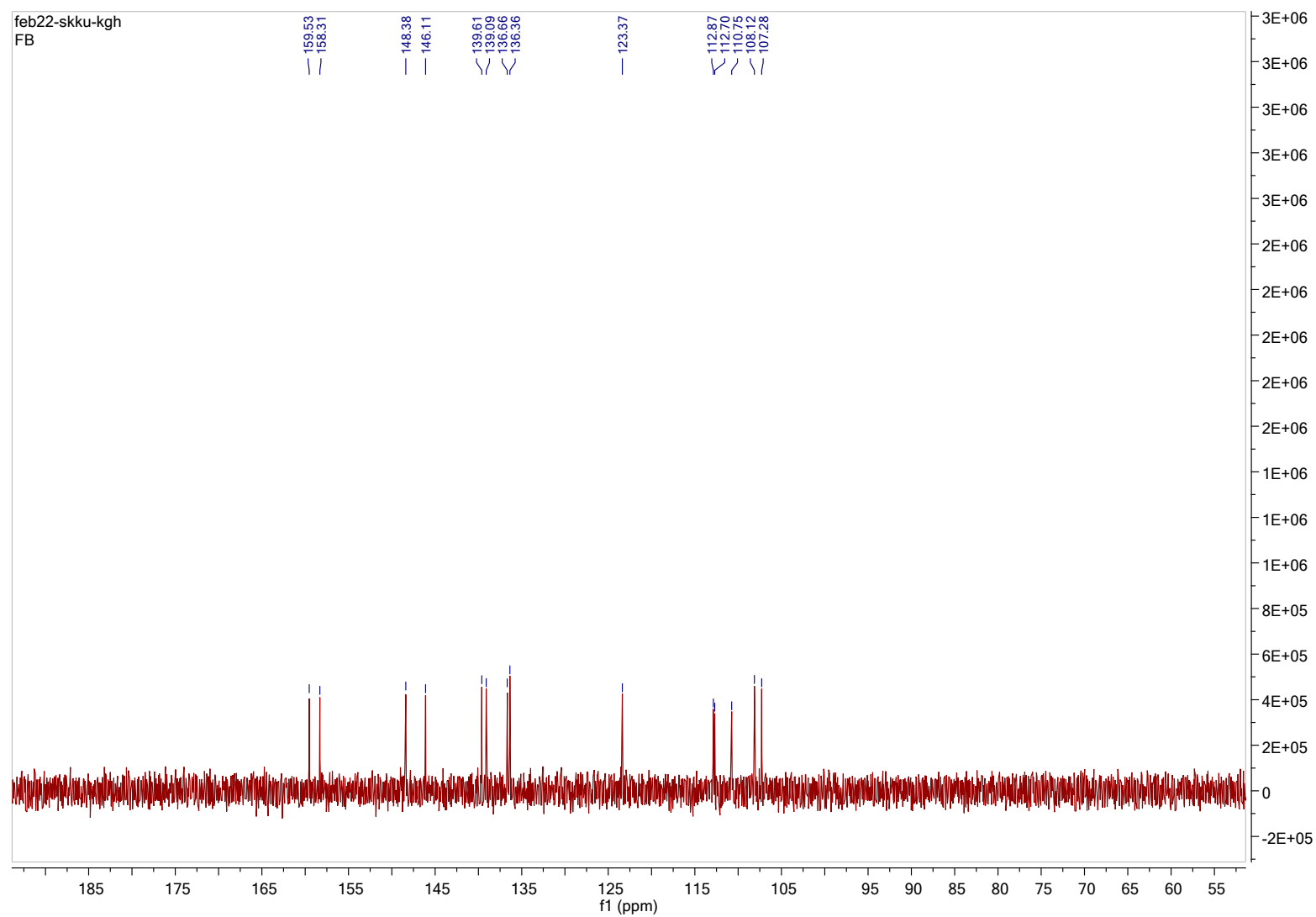


Figure S4. HMBC spectrum of terminalin (DMSO- d_6)

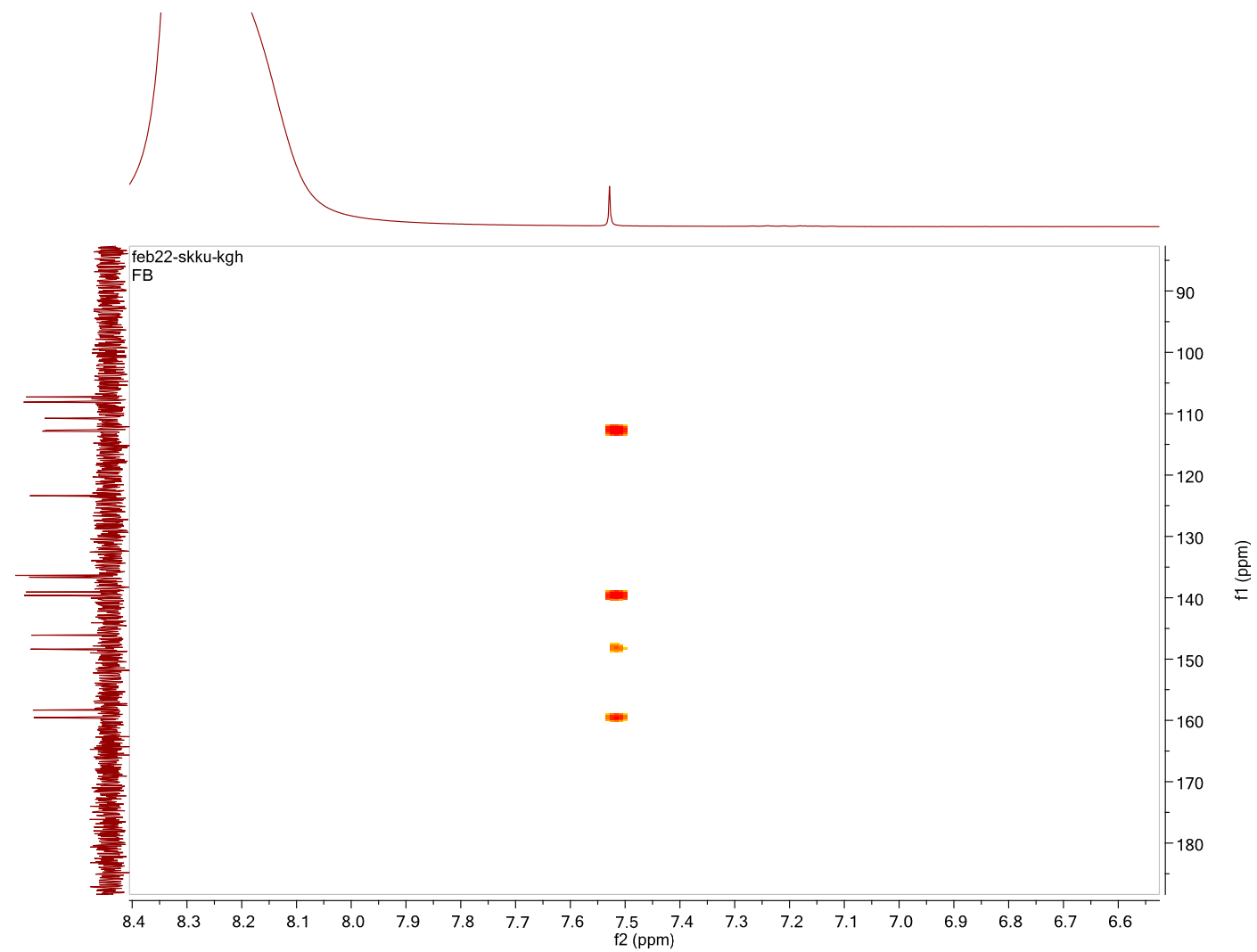


Figure S5. D-HMBC spectrum of terminalin (DMSO- d_6)

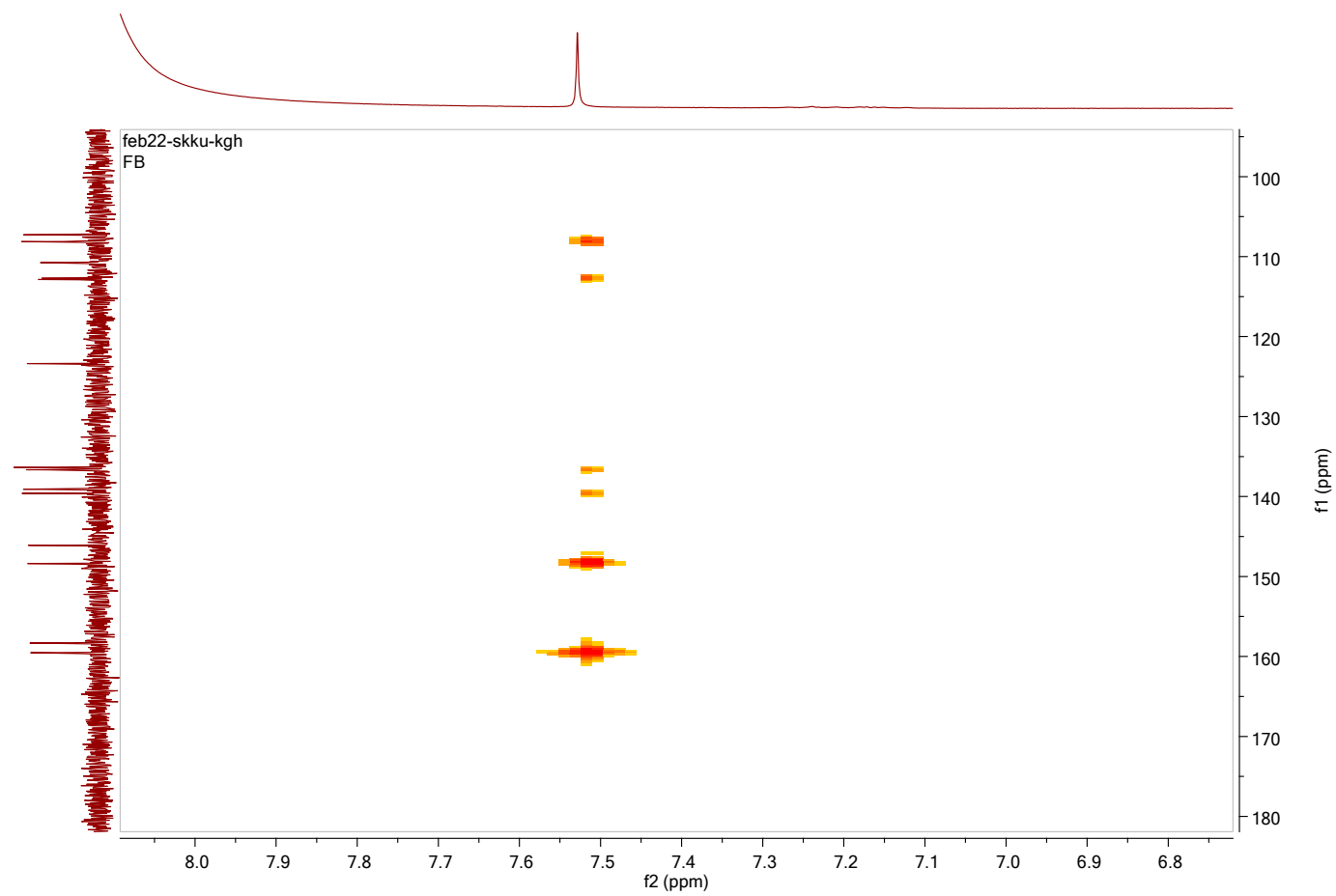


Figure S6. UV spectrum of terminalin

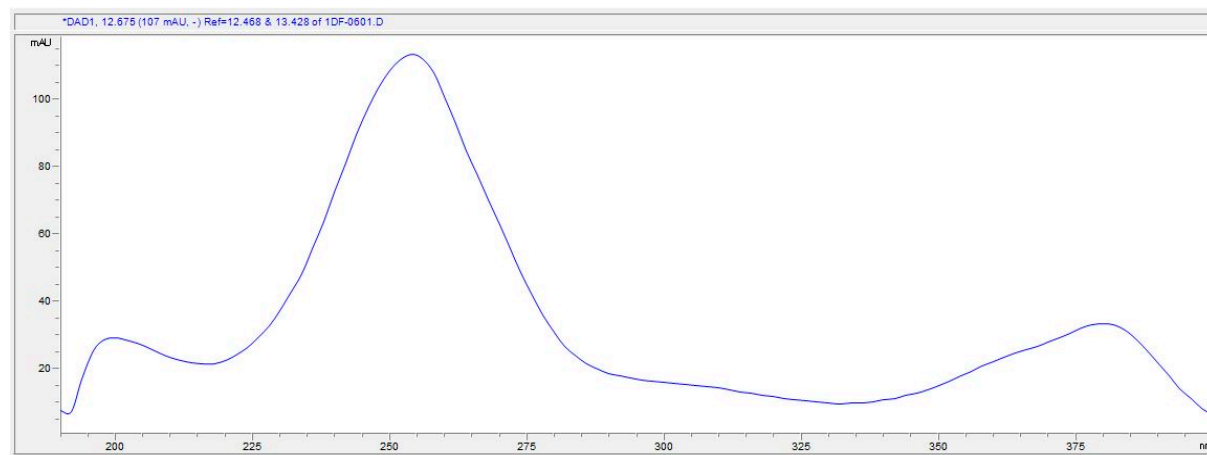


Figure S7. LC/MS chromatogram of the crude extract (detected at 230 nm)

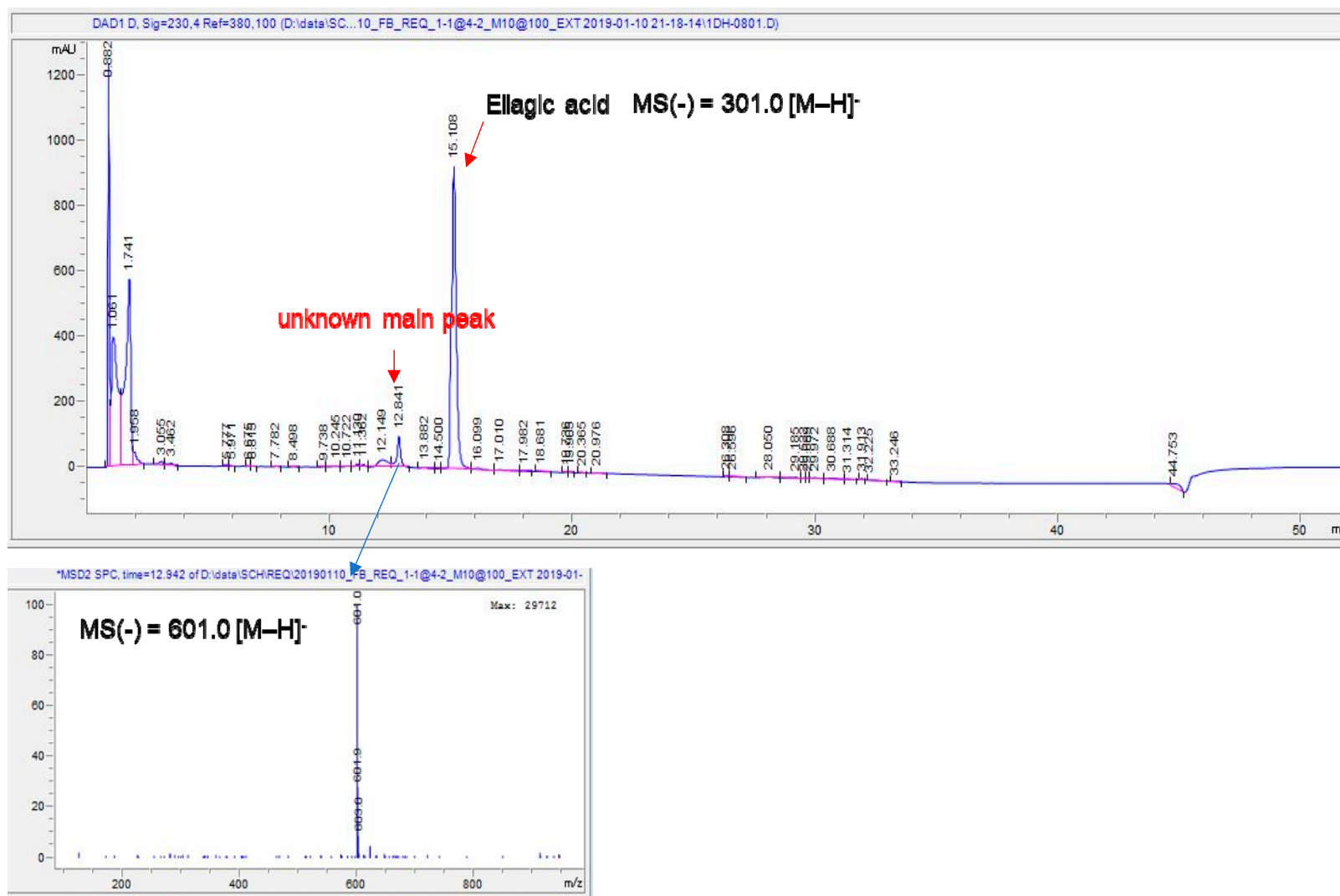


Figure S8. Effects of terminalin on C2C12 muscle cells. Differentiated C2C12 muscle cells were incubated with terminalin, control (0.1% dimethyl sulfoxide), AICAR (positive control; AMPK activator) or insulin (positive control) for 6 h (control, terminalin and AICAR) or 1 h (insulin), and western blotting was performed.

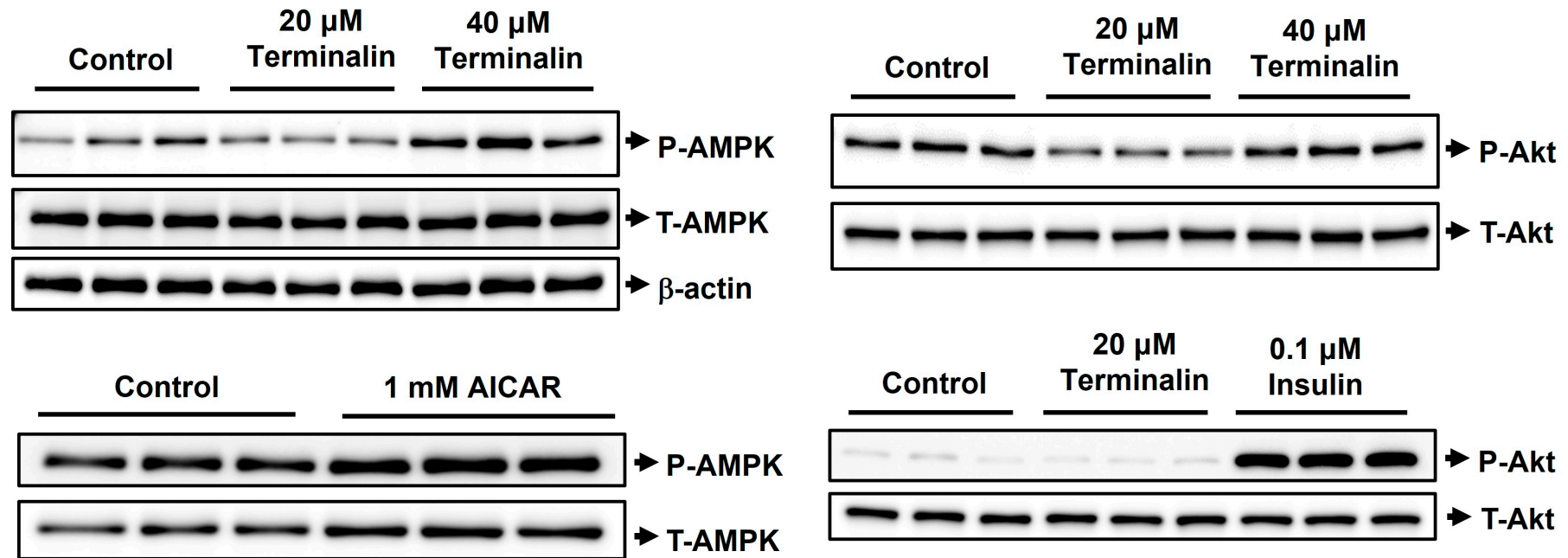


Table S1. LC-MS condition for crude extract

Column	Kinetax C18 column (2.1 × 100 mm, 5 µm; Phenomenex)		
Flow rate	0.3 mL/min		
Injection volume	15 µL		
Column temperature	25°C		
Mobile solvent	A: 0.1% formic acid in water, B: MeOH		
Mobile phase	Time	A (%)	B (%)
	0	90	10
	30	0	100
	31	0	100
	41	0	100
	42	90	10
	52	90	10